526 Rec'd PCT/PTO 10 JUL 2001

FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		4121-126
		U S APPLICATION NO (If known, see 37 CFR 1 5) 09/889182
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE00/00079	11 January 2000	11 January 1999

TITLE OF INVENTION

SELECTION OF MONOCLONAL ANTIBODIES

APPLICANT(S) FOR DO/EO/US

Frank Breitling, Annemarie Poustka and Gerard Moldenhauer

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).*(**Unsigned**)

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☒ A small entity statement.

16. ☐ Other items or information: EPO Search Report in German

NOTE: This application is being filed with an unsigned Oath or Declaration under the provisions of 37 CFR § 1.53 in order that applicants may secure a filing date of July 10, 2001. Upon receipt of a "Notice to File Missing Parts - Filing Date Granted," a executed Declaration and Power of Attorney will be forwarded. The undersigned agent affirmatively states that she has been duly authorized and appointed to file this application on behalf of the applicants and applicants' assignees, and that the Declaration and Power of Attorney to be filed hereafter will confirm the undersigned agent's authorization and appointment. Applicants are considered a small entity and assignee Deutsches Krebsforschungszentrum is also considered a small entity within the meaning of 37 CFR § 1.9.

09/889182

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $860	JC18 Rec'd PCT/PTO 10 JUL 2001	
International preliminary examination fee paid to USPTO (37 CFR 1.482) $0.00		
No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $0.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $0.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	20-20 =	0	X **$18.00**	$	
Independent Claims	2- 3 =	0	X **$80.00**	$	
Multiple dependent claim(s) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				860.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1 27, 1 28).				$ 430.00	
SUBTOTAL =				$ 430.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEE ENCLOSED =				$ 430.00	
				Amount to be: refunded	$
				Charged	$

a. ☒ A check in the amount of $430.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.

Marianne Fuierer

MARIANNE FUIERER
Registration No. 39,983

SEND ALL CORRESPONDENCE TO:

Steven J. Hultquist
Intellectual Property/Technology Law
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Research Triangle Park, NC 27709

09/889182

JC18 Rec'd PCT/PTO 10 JUL 2001

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO $860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $0.00		
No International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $0.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1000.00		
International preliminary examination fee paid to USPTO (37 CFR 1 482) and all claims satisfied provisions of PCT Article 33(2)-(4). $0 00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	20-20 =	0	X **$18.00**	$	
Independent Claims	2- 3 =	0	X **$80.00**	$	
Multiple dependent claim(s) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				860.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 430.00	
SUBTOTAL =				$ 430.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 430.00	
Fee for recording the enclosed assignment (37 CFR 1 21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEE ENCLOSED =				$ 430.00	
				Amount to be: refunded	$
				Charged	$

a. ☒ A check in the amount of $430.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-3284. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not yet been met, a petition to revive (37 CFR 1.127(a) or (b)) must be filed and granted to restore the application to pending status.

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OIPE JC173 JAN 03 2002 PATENT & TRADEMARK OFFICE

JC14 Rec'd PCT/PTO 03 JAN 2002

PCT

4121-126
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	**BREITLING, et al.**
Application No.:	**09/889,182**
International Application No.:	**PCT/DE00/00079**
Priority Date Claimed:	**11 January 2000 and 11 January 1999 (German Appl. No. 199 00 635.0)**
Title:	**SELECTION OF MONOCLONAL ANTIBODIES**

23448
PATENT & TRADEMARK OFFICE

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FIRST CLASS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to the Commissioner for Patents, Washington, DC 20231, and First Class Mailed under the provisions of 37 CFR 1.8.

Lee Ann Brown

Lee Ann Brown

November 14, 2001
Date of Mailing

SUPPLEMENTAL PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified national phase patent application, please amend the application, as follows:

In the Specification

Please insert on page 1 between the title of the application and the first paragraph the following new paragraph:

OIPE
JAN 0 3 2002
PATENT & TRADEMARK OFFICE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is filed under the provisions of 35 U. S.C. §371 and claims the priority of International Patent Application No. PCT/DE00/00079 filed January 11, 2000, and which in turn claims priority of German Patent Application No. 199 00 635.0 filed January 11, 1999.

REMARKS

This claim to priority is being filed before the above-identified application meets all requirements under 35 U.S.C. §371(b).

Respectfully submitted,

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Attorney for Applicants

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09/889182
JC18 Rec'd PCT/PTO 10 JUL 2001

4121-126
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	**BREITLING, et al.**
Application No.:	New U.S. National Stage Application of PCT International Application No. PCT/DE00/00079
International Filing Date:	11 January 2000
Priority Date Claimed:	11 January 1999 (German Appl. No. 199 00 635.0)
U.S. National Phase Filing Date:	Date of mailing identified below
Title:	**SELECTION OF MONOCLONAL ANTIBODIES**

EXPRESS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to the Commissioner for Patents, Box Patent Application, Washington, DC 20231, and Express Mailed under the provisions of 37 CFR 1 10

Blake Crouch
Name of Person Mailing This Document

Signature

July 10, 2001
Date

EL666414295US
Express Mail Label Number

PRELIMINARY AMENDMENT

Commissioner for Patents
BOX PATENT APPLICATION
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified new national phase patent application, please amend the application, as follows:

In the Specification

On the bottom of page 4 and top of page 5, please replace the paragraph with the following paragraph:

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 3 from nucleotide 682-1782) and amino acid sequences (SEQ ID. NO: 4) of the antibody binding protein are given between nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 1 from nucleotide 737-1420) and amino acid sequences (SEQ ID NO: 2)of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA (SEQ ID NO: 5 from nucleotide 682-1431) and amino acid sequences (SEQ ID NO: 6) of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

In the Claims

Please amend claims 1-20 to read as follows:

1. A method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies to

antigens, wherein the antibody binding proteins are inserted in the hybridoma cells via the myeloma cells or in the hybridoma cells via the expression vectors coding therefor.

2. The method according to claim 1, wherein the antibody binding protein comprises a signal peptide, an antibody binding site independent of the antibody specificity and a membrane anchor.

3. The method according to claim 2, wherein the antibody binding protein comprises an Fc binding protein or portions thereof.

4. The method according to claim 2, wherein the antibody binding protein comprises a combination of Fc binding proteins or portions thereof.

5. The method according to claim 4, wherein the Fc binding protein is selected from the group consisting of CD16, CD32 and CD64.

6. The method according to claim 2, wherein the antibody binding protein comprises an antibody binding domain of proteins selected from the group consisting of A, G, L and LG.

7. The method according to claim 2, wherein the antibody binding protein comprises a combination of a signal peptide selected from the group consisting of a signal peptide of a mouse Ig cappa chain, and a signal peptide of a mouse MHC-class I k(k) molecule; an antibody binding site of a protein selected from the group consisting of protein A, G, L, and LG; and a transmembrane domain selected from the group consisting of PDGFR and CD52.

8. The method according to claim 7, wherein the antibody binding protein is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6.

9. The method according to claim 1, wherein the hybridoma cells (over)express Rag1 and/or Rag2.

10. The method according to claim 1, wherein the antigens originate from an antigen library.

11. The method according to claim 1, wherein the antigens are bound to a carrier.

12. The method according to claim 11, wherein the carrier comprises magnetobeads.

13. The method according to claim 7, wherein the antigens comprise a fluorescence or biotin labeling.

14. The method according to claim 13, wherein the fluorescence labeling comprises FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin.

15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide selected from the group consisting of a mouse Ig cappa chain and a mouse MHC-class I k(k) molecule, an antibody binding site of proteins selected from the group consisting of A, G, L and LG and a transmembrane domain selected from the group consisting of PDGFR and CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID.NO 4 and SEQ ID NO:6 or an amino acid sequence differing therefrom by one or more amino acids.

17. DNA coding for the antibody binding protein according to claim 16, comprising:
    (a) the DNA of an antibody binding protein selected from the group consisting of SEQ ID NO: 1 from nucleotide 737-1420, SEQ ID NO: 3 from nucleotide 682-1782, and SEQ ID NO: 5 from nucleotide 682-1431

[of figure 1, 2 or 3], a DNA differing therefrom by one or more base pairs, or

(b) a DNA related to the DNA of (a) via the degenerated code.

18. An expression vector, coding for the DNA according to claim 17.

19. Cells containing the expression vector according to claim 18.

20. An antibody directed against the antibody binding protein according to claim 16.

REMARKS

A marked-up version of amended paragraph in the specification and amended claims 1-20 are included herewith in Appendix A.

It is requested that the examination and prosecution of this application proceed on the basis of the English translation of the PCT International application included herewith and these amended claims 1-20.

Respectfully submitted,

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APPENDIX A

In the Specification

On the bottom of page 4 and top of page 5, replace the paragraph with the following paragraph:

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 3 from nucleotide 682-1782) and amino acid sequences (SEQ ID. NO: 4) of the antibody binding protein are given between nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA (SEQ ID NO: 1 from nucleotide 737-1420) and amino acid sequences (SEQ ID NO: 2)of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA (SEQ ID NO: 5 from nucleotide 682-1431)and amino acid sequences (SEQ ID NO: 6) of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

In the Claims

5. The method according to claim [3 or] 4, wherein the Fc binding protein is selected from the group consisting of CD16, CD32 [or] and CD64.

6. The method according to claim 2 [any of claims 2 to 5], wherein the antibody binding protein comprises an antibody binding domain of proteins selected from the group consisting of A, G, L and [or] LG.

7. The method according to claim 2, wherein the antibody binding protein comprises a combination of a [the] signal peptide selected from the group consisting of a signal peptide of a mouse Ig cappa chain, and a signal peptide of a mouse MHC-class I k(k) molecule; an antibody binding site of a protein[s] selected from the group consisting of protein A, G, L, and [or] LG; and [the] a transmembrane domain selected from the group consisting of PDGFR [or] and CD52.

8. The method according to claim 7, wherein the antibody binding protein is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6. [that of figure 1, figure 2 or figure 3.]

9. The method according to claim 1 [any of claims 1 to 8], wherein the hybridoma cells (over)express Rag1 and/or Rag2.

10. The method according to claim 1 [any of claims 1 to 9], wherein the antigens originate from an antigen library.

11. The method according to claim 1 [any of claims 1 to 10], wherein the antigens are bound to a carrier.

13. The method according to claim 7, [any of claims 1 to 10], wherein the antigens comprise a fluorescence or biotin labeling.

15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide selected from the group consisting of a mouse Ig cappa chain [or] and a mouse MHC-class I k(k) molecule, an antibody binding site of proteins selected from the group consisting of A, G, L [or] and LG and a [the] transmembrane domain selected from the group consisting of PDGFR [or] and CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises an [the] amino acid sequence selected from the group consisting of [figure 1, figure 2 or figure 3] SEQ ID NO: 2, SEQ ID.NO 4 and SEQ ID NO:6 or an amino acid sequence differing therefrom by one or more amino acids.

17. DNA coding for the antibody binding protein according to claim 16, comprising:

(a) the DNA of an antibody binding protein selected from the group consisting of SEQ ID NO: 1 from nucleotide 737-1420, SEQ ID NO: 3 from nucleotide 682-1782, and SEQ ID NO: 5 from nucleotide 682-1431 [of figure 1, 2 or 3], a DNA differing therefrom by one or more base pairs, or

(b) a DNA related to the DNA of (a) via the degenerated code.

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Selection of Monoclonal Antibodies

The present invention relates to a method of selecting monoclonal antibodies and to means which can be used therefor.

The production of monoclonal antibodies is based on a method developed by Kohler and Milstein. According to this method B lymphocytes are fused with myeloma cells so as to obtain antibody-producing hybridoma cells. Such a method comprises major drawbacks. In particular, it is time-consuming and expensive to select antibodies, since this calls for separate culturing of hybridoma cells. Due to the latter only a limited number of hybridoma cells is detected and thus not all of the antibodies can be selected, this being a drawback in particular when antibodies with maximum affinity for an antigen shall be selected.

It is thus the object of the present invention to provide a product by which monoclonal antibodies can be produced, the above drawbacks being avoided.

According to the invention this is achieved by the subject matters defined in the claims.

The present invention is based on Applicant's insights that monoclonal antibodies on the cell surface of hybridoma cells can be presented by means of an antibody binding protein. He realized that monoclonal antibodies can be selected by this without hybridoma cells having to be cultured separately. He also realized that monoclonal antibodies can be selected

with respect to a determined and many (un)determined antigens of an antigen library. Furthermore, he found that monoclonal antibodies can also be selected with respect to their affinity intensity for certain antigens.

According to the invention Applicant's insights are used to provide a method of selecting monoclonal antibodies. Such a method comprises fusing B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and binding of the antibodies to antigens.

The expression "B lymphocytes" comprises B lymphocytes of any kind and origin. They may also concern precursors of B lymphocytes. The B lymphocytes may originate from animals, such as mice, rats, rabbits, etc., or humans. The B lymphocytes may also originate from a healthy or diseased organism. It is favorable for them to originate from an immunized organism. It is particularly favorable for the B lymphocytes to code for human antibodies or portions thereof. If B lymphocytes from animals are concerned, this can be achieved when the animals are transgenic for the human antibodies or portions thereof. Such animals can be produced by common methods, it being an obvious thing to introduce the genes for the human antibodies or the portions thereof into embryonal stem cells from which the animals are then generated. B lymphocytes and their precursors may be provided by common methods.

The expression "myeloma cells" comprises myeloma cells of any kind and origin. They may also concern precursors of myeloma cells. Furthermore, the myeloma cells may originate from animals, such as mice, rats, rabbits, etc., or humans.

Preferred myeloma cells are descendents from the mouse strains P3K, P3-X63.Ag8, X63.Ag8.653, NSO/1, Sp2/O-Ag14 and FO, the rat strains Y3-Ag1.2.3, YB2/0 and IR9834, and the human strains U266, SK007 and Karpas 707. Myeloma cells and their precursors can be provided by common methods.

The expression "antibody-producing hybridoma cells" comprises cells which form by fusion of B lymphocytes and myeloma cells and produce antibodies. Corresponding reference is made to the statements on B lymphocytes and myeloma cells. Hybridoma cells may include animal and/or human nucleic acids and/or proteins. Hybridoma cells can be cultured by common methods. It may also be favorable for the hybridoma cells to (over)express recombinases, e.g. Rag1 or Rag2, and/or mutases. This can be achieved by transfection of the hybridoma cells with corresponding expression vectors. The person skilled in the art knows such expression vectors.

The term "fusion of B lymphocytes with myeloma cells" concerns any method by means of which these cells may be fused. A method is favorable in which the cells are fused via polyethylene glycol. Reference is made to the examples.

The term "binding of the antibodies to antigens" concerns any method by which the antibodies expressed on the cell surface of the hybridoma cells can bind to antigens. The antigens can be bound to carriers, e.g. magnetobeads. They can also be labeled, e.g. fluorescence-labeled. For example FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin offer themselves as fluorescence markers. The antigens may also be coupled to biotin. Bound antigens may be detected by common methods, e.g. FACS analysis whereby the corresponding

antibodies are also detected. Reference is made to the examples.

The expression "antibody binding protein" comprises any protein which may bind an antibody and present it on the cell surface of hybridoma cells. In particular, the protein may have a signal peptide, an antibody-binding site independent of the specificity of the antibody and a membrane anchor. Examples of such a protein are natural Fc binding proteins, such as CD16, CD32 and CD64. The protein may comprise a combination of a signal peptide, an antibody binding site and a membrane anchor, which does not occur in nature. Such a combination may comprise portions of natural Fc binding proteins. Furthermore, as a signal peptide it may have one of a mouse Ig kappa chain or a mouse MHC-class I k(k) molecule, as a membrane anchor it may include a transmembrane domain of PDGRF or CD52 and as an antibody binding site it may comprise an antigen binding domain of a bacterial protein, such as protein A, protein G, protein L or protein LG. It may be favorable for the combination to comprise several signal peptides, antibody binding sites and/or membrane anchors. It may be particularly favorable for the antibody binding protein, in particular the antibody binding domain of the bacterial proteins, to have codons which are optimized for expression in mammalian cells. A person skilled in the art knows which codons are concerned here.

Preferred antibody binding proteins are shown in figures 1 to 3. The antibody binding protein of figure 1 comprises the signal peptide of a mouse MHC class I k(k) molecule, four antibody binding domains of the L protein and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are given between

nucleotide numbers 682-1782. The antibody binding protein of figure 2 comprises the signal peptide of a mouse Ig kappa chain, two antibody binding sites of the G protein and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are indicated between nucleotide numbers 737-1420. The antibody binding protein of figure 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibodies binding sites of the G protein and the transmembrane domain of PDGFR. The DNA and amino acid sequences of the antibody binding protein are given between nucleotide numbers 682-1431. The antibody binding sites of all three antibody binding proteins have, on a DNA level, codons which are optimized for expression in mammalian cells.

An antibody binding protein of figures 1, 2 or 3 may have an amino acid sequence which differs from the amino acid sequence in figure 1, 2 or 3 by one or more amino acids. The differences may lie in additions, deletions, substitutions and/or inversions of individual amino acids. However, the DNA of this antibody binding protein hybridizes with the DNA indicated in figure 1, 2 or 3. The term "hybridizing" refers to hybridization under common conditions, in particular at 20°C below the melting point of the DNA. Furthermore, the antibody binding protein having the modified amino acid sequence comprises whole or partial functions which can be compared with those of the antibody binding protein of figure 1, 2 or 3.

Another subject matter of the present invention relates to a nucleic acid which codes for an above antibody binding protein. The nucleic acid may be an RNA or a DNA. Preferred is a DNA which comprises the following:

(a) the DNA of an antibody binding protein of figure 1, 2 or 3, a DNA differing therefrom by one or more base pairs, or

(b) a DNA related to the DNA from (a) by the degenerated genetic code.

The term "a DNA differing by one or more base pairs" comprises any DNA coding for an antibody binding protein of figure 1, 2 or 3, which hybridizes with the DNA of figure 1, 2 or 3. The differences may lie in additions, deletions, substitutions and/or inversions of individual base pairs. As to the term "hybridizing" reference is made to the above explanations.

A DNA according to the invention may be present as such or in combination with any other DNA. In particular, a DNA according to the invention, which codes for an antibody binding protein, may be present in an expression vector. The person skilled in the art is familiar with examples thereof. In the case of an expression vector for *E. coli* these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b and pQE-8. For the expression in yeast, e.g. pY100 and Ycpad1 have to be mentioned while e.g. pKCR, pEFBOS, pCDM8 and pCEV4 have to be indicated for the expression in animal cells. The bacculovirus expression vector pAcSGHisNT-A is particularly suitable for the expression in insect cells.

The person skilled in the art knows how to insert the DNA according to the invention in an expression vector. He also knows that this DNA can be inserted in combination with a DNA coding for another protein or peptide, so that the DNA according to the invention can be expressed in the form of a fusion protein.

Preferred expression vectors which contain a DNA according to the invention are shown in figures 1 to 3. The expression vectors pSEX11L4, pSEX11G2* and pSEX15G2 are concerned. They were deposited with the DSMZ (*Deutsche Sammlung für Mikroorganismen und Zellkulturen* [German-type collection of microorganisms and cell cultures]) on December 14, 1998. In particular, pSEX11L4 was deposited under DSM 12580, pSex11G2* was deposited under DSM 12581 and pSEX15G2 was deposited under DSM 12582.

The person skilled in the art is familiar with suitable cells to express a cDNA according to the invention, which is present in an expression vector. Examples of such cells comprise the E. *coli* strains XL-1 Blue, Top 10 F, HB101, DH5alpha, x1776, JM101, JM 109, BL21 and SG 13009, the yeast strain *Saccharomyces cerevisiae* and *Pichia pastoris*, the animal cells L, NIH 3T3, FM3A, CHO, COS, Vero, HeLa, myeloma and hybridoma cells as well as the insect cells sf9.

The person skilled in the art also knows conditions of culturing transformed or transfected cells. He is also familiar with methods of isolating and purifying the protein or fusion protein expressed by the cDNA according to the invention.

Another subject matter of the present invention relates to an antibody directed against an above protein or fusion protein. Such an antibody may be prepared by common methods. It may be polyclonal or monoclonal. For its preparation it is favorable to immunize animals - in particular rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above (fusion) protein or with fragments thereof. Further "boosters" of the animals can be effected

with the same (fusion) protein or with fragments thereof. The polyclonal antibody may then be obtained from the animal serum or egg. For the preparation of the monoclonal antibody, animal spleen cells are fused with myeloma cells.

Another subject matter of the present invention is a kit. Such a kit comprises one or more of the following components:

(a) a DNA according to the invention,
(b) a cell expressing a DNA according to the invention,
(c) an antibody binding protein according to the invention,
(d) an antibody according to the invention, and
(e) common auxiliary substances such as carriers, buffers, solvents, controls, markers, detection reagents for components (a) - (d).

One or more representatives of the individual components may be present. As to the individual terms reference is made to the above statements. They apply here analogously.

The present invention distinguishes itself in that antibodies produced by hybridoma cells are presented on the cell surface of the hybridoma cells. This is done via an antibody binding protein. Such a protein may be introduced into the hybridoma cells via the myeloma cells used for the production of the hybridoma cells. The antibody binding protein may also be introduced into the hybridoma cells via an expression vector coding for it.

By means of the present invention it is possible to select antibodies. This can be done without much expenditure, since hybridoma cells do not have to be cultured separately. Complex mixtures of hybridoma cells can rather be used

directly for selecting antibodies. Antibodies can also be selected for their affinity intensity for certain antigens. The present invention is also suitable for selecting antibodies of hybridoma cell libraries, not only with respect to a determined antigen but also as regards many (un)determined antigens of antigen libraries.

Thus, the present invention provides products by which it is possible to avoid *inter alia* major problems as regards time and cost which have occurred in the selection of monoclonal antibodies thus far.

Brief description of the drawings:

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Fig. 1 shows the expression vector pSEX11L4 according to the invention (figure 1A) which codes for an antibody binding protein (figure 1(B)). Reference is made to the above explanations.

Fig. 3 shows the expression vector pSEX11G2* according to the invention (figure 2(A)), which codes for an antibody binding protein (figure 2(B)). Reference is made to the above explanations.

Figure 3 shows the expression vector pSEX15G2 according to the invention (figure 3(A)), which codes for an antibody binding protein (figure 3(B)). Reference is made to the above explanations.

The present invention is explained by the below examples.

Example 1: Preparation of myeloma cells which express an antibody binding protein on their cell surface

(A) Transient expression

Cells of the myeloma cell line X63-Ag8.653 are used. These cells (10^7) are transfected with 20-40 µg of the expression vector SEX11G2* according to the invention (cf. figure 2). Electroporation is carried out as transfection technique, which comprises two pulses of 2 ms at 500 V. The cells are incubated for 48 h at 37°C and 5-7.5 % Co_2 in RPMI medium which contains 10 % FCS. Thereafter, the cells are washed with cold DPBS + 0.1 % Na azide before they are incubated for 45 minutes at 0°C with DPBS + 0.1 % Na azide plus 25 µg/ml goat anti-calf antibody (FITC-labeled; GAB-FITC, Dianova company). Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 1 µg/ml propidium iodide and subjected to FACS analysis following excitation with blue light.

It shows that the transfected myeloma cells have a green fluorescence which is due to the transient expression of an antibody binding protein on the cell surface of the myeloma cells.

(B) Stable expression

The myeloma cells obtained under (A) are subjected to G418 selection for 14-24 days before they are incubated using GAB-FITC and subjected to FACS analysis as described under (A). Myeloma cells which have a strong green fluorescence are subject to further G418 selection rounds or runs.

The myeloma cell line X63-Ag8.653.3 is obtained which stably expresses an antibody binding protein on its cell surface.

Example 2: Production of hybridoma cells which express on their cell surface antibodies by means of an antibody binding protein

(A)

10 Balb/c mice are immunized subcutaneously in each case with 100 µg killed *Helicobacter pylori* bacteria in complete Freund's adjuvant, which contains killed *Mycobacter tuberculosis* bacteria. After 4 or 7 weeks, an intraperitoneal booster injection with 100 µg killed *Helicobacter pylori/Mycobacter tuberculosis* bacteria is given. 100 µl blood serum are withdrawn from the mice before each immunization and after the last immunization, and the antigen-specific immune response of the mouse is tested in a Western blot. A degradation of bacterial whole protein of *Helicobacter pylori* and/or *Mycobacter tuberculosis* is used as antigen. The detection of bound mouse antibodies is made by an peroxidase-conjugated goat anti-mouse antibody (Dianova company). The spleen of mice having a marked antigen-specific immune response is removed and the lymphocytes are fused with cells of the myeloma cell line X63-Ag8.653.3 of Example 1 (B). The fusion is made by means of polyethylene glycol (cf. Goding, J.W., Cell Biology, Biochemistry and Immunology, 3rd edition (1996), Verlag Academic Press Limited, 24-28). Hybridoma cells are obtained. They are incubated in HAT medium at 37°C for 10 to 12 days. The hybridoma cell library 2A is obtained.

Hexapeptides with N-terminal biotin are synthesized. The peptides correspond to the 6C-terminal amino acids of 101 or 118 gene products of *Helicobacter pylory* or *Mycobacter*

tuberculosis. 10^3 cells of the hybridoma cell library 2A are also washed with cold DPBS + 0.1 % Na azide and incubated for 45 minutes at 0°C with DPBS + 0.1 % Na azide + 10 µg/ml of the above biotin-labeled peptides. The cells are washed with cold DPBS + 0.1 % Na azide and incubated for 45 minutes at 0°C with 10 µg/ml streptavidine FITC. Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 1 µg/ml propidium iodide and subjected to FACS analysis after excitation with blue light.

It shows that the hybridoma cells have a green fluorescence. This fluorescence is due to the expression of antibodies on the cell surface of the hybridoma cells. Further studies show that the antibodies have an anti-*Helicobacter pylori* or *Mycobacter tuberculosis* activity.

(B)

Cells of the hybridoma cell line U98/6 which produce a mouse anti-urokinase antibody are used. These cells (10^7) are transfected with 20-40 µg of the pSEX11G2* expression vector according to the invention (cf. figure 2). Electroporation is carried out as a transfection technique, which comprises two pulses of 2 ms at 400 V. The cells are incubated for 48 h in incomplete AIM V-medium at 37°C and 5-7.5 % Co_2. Thereafter, the cells are washed with cold DPBS + 0.1 % Na azide before they are incubated at 0°C for 45 minutes with DPBS + 0.1 % Na azide + 10 µg/ml urokinase biotin. Having been washed with DPBS + 0.1 % Na azide, the cells are incubated in DPBS + 0.1 % Na azide + 10 µg/ml streptavidine FITC and subjected to FACS analysis after excitation with blue light.

It shows that the transfected hybridoma cells have a green fluorescence. This fluorescence is due to the expression of

antibodies on the cell surface of the hybridoma cells. Further studies show that the antibodies have anti-urokinase activity.

The resulting hybridoma cells are subjected to G418 selection for 14 to 24 days before they are again incubated with urokinase-biotin and streptaividine-FICS and subjected to FACS analysis as described above. Hybridoma cells which have a strong green fluorescence are subjected to further G418 selection rounds.

The hybridoma cell line U98/6.3.3 is obtained. It stably expresses antibodies on its cell surface.

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Example 3: Selection of monoclonal antibodies which are expressed on the cell surface of hybridoma cells by means of an antibody binding protein

10^3 cells of the hybridoma cell line U98/6.3.3 of Example 2 (B) are mixed with 10^7 cells of the hybridoma cell line DOB.L1.3. The latter hybridoma cell line produces an antibody recognizing the C terminus of the human HLA-DO-ß chain. It is expressed on the cell surface by means of an antibody binding protein the same as that in the hybridoma cell line U98/6.3.3 of Example 2(B). The cell mixture is washed with cold DPBS + 0.1 T Na azide and incubated at 0°C for 45 minutes with DPBS + 0.1 % Na azide + 10 µg/ml urokinase biotin. Having been washed with DPBS + 0.1 % Na azide, the cell mixture is incubated in DPBS + 0.1 % Na azide + 10 µg/ml streptavidine FITC and supplied to a FACS sorter following excitation with blue light.

Hybridoma cells with green fluorescence are selected. In further studies, they show an anti-urokinase activity. The hybridoma cell lines U98/6.3.3 S1-S50 are obtained.

Example 4: Production and purification of an antibody binding protein according to the invention

(A)

The DNA of figure 1 between nucleotide numbers 682-1782 is provided with BAMHI linkers, subsequently cleaved using BamHI, and inserted in the pQE-8 expression vector cleaved by BamHI (Qiagen company). The expression plasmid pQE-8/antibody binding protein is obtained. Such a plasmid codes for a fusion protein comprising 6 histidine residues (N terminus partner) and the antibody binding protein of fig. 1 according to the invention (C terminus partner). pQE-8/antibody binding protein is used for transforming *E. coli* SG 13009 (cf. Gottesman, S. *et al.*, J. Bacteriol. 148, (1981), 265-273). The bacteria are cultured in an LB broth with 100 µg/ml ampicillin and 25 µg/ml kanamycin and induced with 60 µM isopropyl-ß-D-thiogalactopyranoside (IPTG) for 4 h. Lysis of the bacteria is achieved by the addition of 6 M guanidine hydrochloride. Thereafter, chromatography (Ni-NTA resin) is carried out with the lysate in the presence of 8 M urea in accordance with the instructions from the manufacturer of the chromatography material (Qiagen company). The bound fusion protein is eluted in a buffer having a pH of 3.5. After its neutralization, the fusion protein is subjected to 18 % SDS polyacrylamide gel electrophoresis and stained with coomassie blue (cf. Thomas, J.O., and Kornberg, R.D., J. Mol. Biol. 149 (1975), 709-733).

It shows that an antibody binding protein (fusion protein) according to the invention can be prepared in highly pure form.

(B)

10^8 cells of the myeloma cell line X63-Ag8.653.3 obtained in Example 1 (B) are washed with PBS, taken up in PBS + 1 % Tween 20 and incubated on ice. Particulate cell components are separated by centrifugation at 30,000 g, and the supernatant is placed on an IgG sepharose column (IgG sepharose 6 Fast Flow Lab Pack from Pharmacia company). Unbound components are removed by washing and the antibody binding protein according to the invention is eluted in acidic pH.

Following its neutralization, the antibody binding protein is subjected to 18 % SDS polyacrylamide gel electrophoresis and stained using coomassie blue (see above).

It showed that an antibody binding protein (fusion protein) according to the invention can be obtained in highly pure form.

Example 5: Preparation and detection of an antibody according to the invention

A fusion protein of Example 4 according to the invention is subjected to 18 % SDS polyacrylamide gel electrophoresis. After staining the gel with 4 M sodium acetate, an about 41 kD band was excised from the gel and incubated in phosphate-buffered common salt solution. Gel pieces are sedimented before the protein concentration of the supernatant is determined by SDS polyacrylamide gel electrophoresis which

is followed by coomassie blue staining. Animals are immunized with the gel-purified fusion protein as follows:

Immunization protocol for polyclonal antibodies in rabbits

35 µg of gel-purified fusion protein in 0.7 ml PBS and 0.7 ml of complete or incomplete Freund's adjuvant were used per immunization:

Day 0: 1st immunization (complete Freund's adjuvant)

Day 14: 2nd immunization (incomplete Freund's adjuvant; icFA)

Day 28: 3rd immunization (icFA)

Day 56: 4th immunization (icFA)

Day 80: bleeding to death.

The rabbit serum is tested in an immunoblot. For this purpose, a fusion protein of Example 4 according to the invention is subjected to SDS polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter (cf. Khyse-Andersen, J., J. Biochem. Biophys. Meth. 10 (1984), 203-209). The Western blot analysis was carried out as described in Bock, C.-T. *et al.*, Virus Genes 8, (1994), 215-229. For this purpose, the nitrocellulose filter is incubated with a first antibody at 37°C for one hour. This antibody is the rabbit serum (1:10000 in PBS). After several wash steps using PBS, the nitrocellulose filter is incubated with a second antibody. This antibody is an alkaline phosphatase-coupled monoclonal goat anti-rabbit IgG antibody (Dianova company) (1:5000) in PBS. 30 minutes of incubation at 37°C are followed by several wash steps using PBS and subsequently by the alkaline phosphatase detection reaction with developer solution (36 µM 5'-bromo-4-chloro-3-indolylphosphate, 400 µM nitro blue tetrazolium, 100 mM

Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) at room temperature until bands are visible.

It shows that polyclonal antibodies according to the invention can be prepared.

Immunization protocol for polyclonal antibodies in chickens

40 µg of gel-purified fusion protein in 0.8 ml PBS and 0.8 ml of complete or incomplete Freund's adjuvant were used per immunization.

Day 0: 1st immunization (complete Freund's adjuvant)
Day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
Day 50: 3rd immunization (icFA)

Antibodies are extracted from egg yolk and tested in a Western blot. Polyclonal antibodies according to the invention are detected.

Immunization protocol for monoclonal antibodies in mice

12 µg of gel-purified fusion protein in 0.25 ml PBS and 0.25 ml of complete or incomplete Freund's adjuvant are used per immunization. The fusion protein is dissolved in 0.5 ml (without adjuvant) in the 4th immunization.

Day 0: 1st immunization (complete Freund's adjuvant)
Day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
Day 56: 3rd immunization (icFA)
Day 84: 4th immunization (PBS)
Day 87: fusion.

Supernatants of hybridomas are tested in a Western blot. Monoclonal antibodies according to the invention are detected.

Amended Claims

1. A method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, the antibodies being presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies to antigens, wherein the antibody binding proteins are inserted in the hybridoma cells via the myeloma cells or in the hybridoma cells via the expression vectors coding therefor.

2. The method according to claim 1, wherein the antibody binding protein comprises a signal peptide, an antibody binding site independent of the antibody specificity and a membrane anchor.

3. The method according to claim 2, wherein the antibody binding protein comprises an Fc binding protein or portions thereof.

4. The method according to claim 2, wherein the antibody binding protein comprises a combination of Fc binding proteins or portions thereof.

5. The method according to claim 3 or 4, wherein the Fc binding protein is CD16, CD32 or CD64.

6. The method according to any of claims 2 to 5, wherein the antibody binding protein comprises an antibody binding domain of proteins A, G, L or LG.

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7. The method according to claim 2, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig cappa chain or a mouse MHC-class I k(k) molecule, an antibody binding site of proteins A, G, L or LG and the transmembrane domain of PDGFR or CD52.

8. The method according to claim 7, wherein the antibody binding protein is that of figure 1, figure 2 or figure 3.

9. The method according to any of claims 1 to 8, wherein the hybridoma cells (over)express Rag1 and/or Rag2.

10. The method according to any of claims 1 to 9, wherein the antigens originate from an antigen library.

11. The method according to any of claims 1 to 10, wherein the antigens are bound to a carrier.

12. The method according to claim 11, wherein the carrier comprises magnetobeads.

13. The method according to any of claims 1 to 10, wherein the antigens comprise a fluorescence or biotin labeling.

14. The method according to claim 13, wherein the fluorescence labeling comprises FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7 and phycoerythrin.

15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig cappa chain or a mouse MHC-class

I k(k) molecule, an antibody binding site of proteins A, G, L or LG and the transmembrane domain of PDGFR or CD52.

16. The antibody binding protein according to claim 15, wherein the antibody binding protein comprises the amino acid sequence of figure 1, figure 2 or figure 3 or an amino acid sequence differing therefrom by one or more amino acids.

17. DNA coding for the antibody binding protein according to claim 16, comprising:
    (a) the DNA of an antibody binding protein of figure 1, 2 or 3, a DNA differing therefrom by one or more base pairs, or
    (b) a DNA related to the DNA of (a) via the degenerated code.

18. An expression vector, coding for the DNA according to claim 17.

19. Cells containing the expression vector according to claim 18.

20. An antibody directed against the antibody binding protein according to claim 16.

Substitute Sheet

Abstract of the Disclosure

The present invention relates to a method of selecting monoclonal antibodies, comprising the fusion of B lymphocytes with myeloma cells to form antibody-producing hybridoma cells, wherein the antibodies are presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and to the binding of the antibodies to antigens. The invention also concerns means usable for this purpose.

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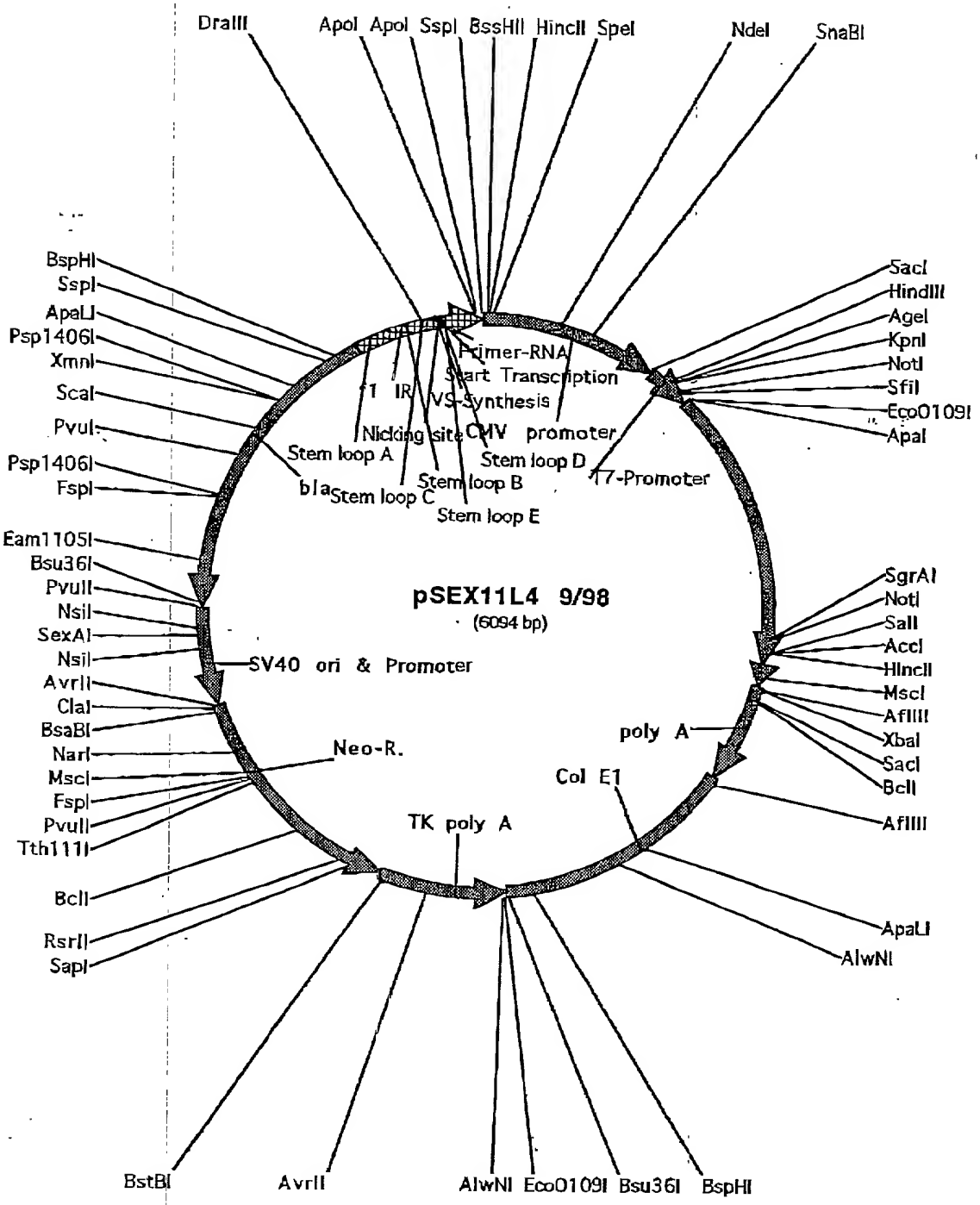
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Stem loop B
Stem loop C
Stem loop D
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Neo-R.
poly A
Col E1
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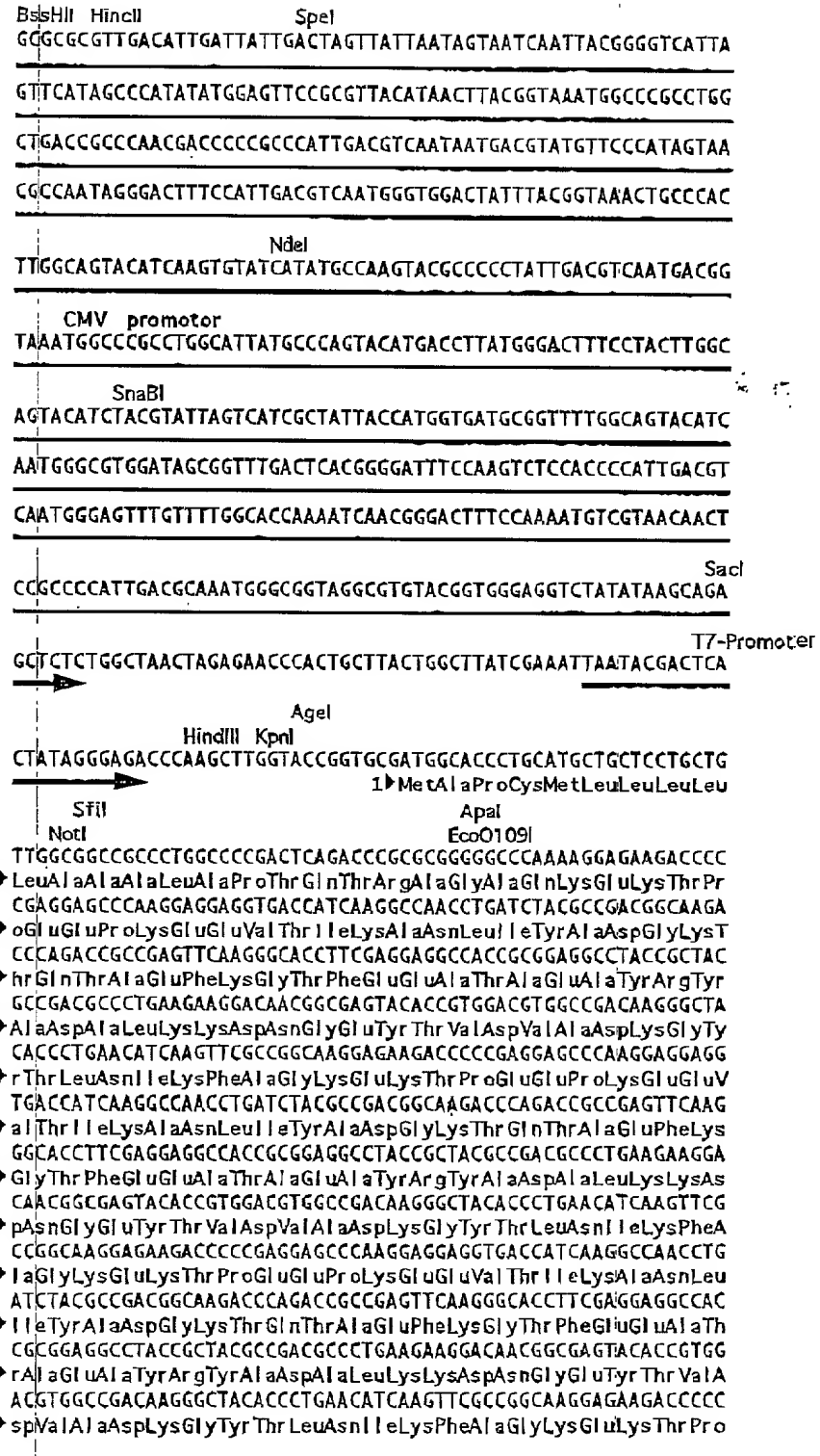
Fig. 1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NdeI
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CMV promotor
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SacI
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T7-Promoter
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HindIII KpnI
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Fig. 1 (cont'd I)

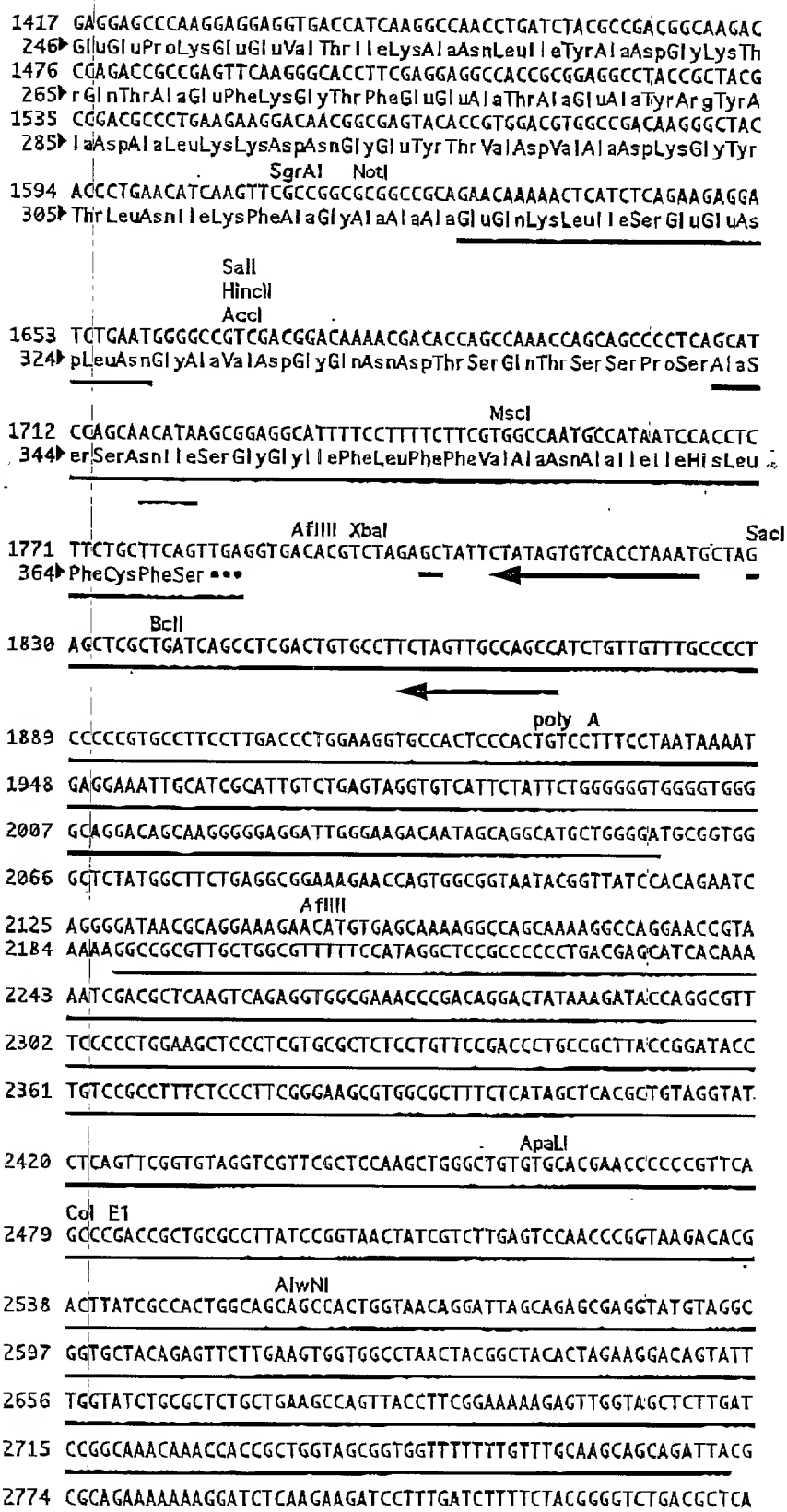

Fig. 1 (cont'd II)

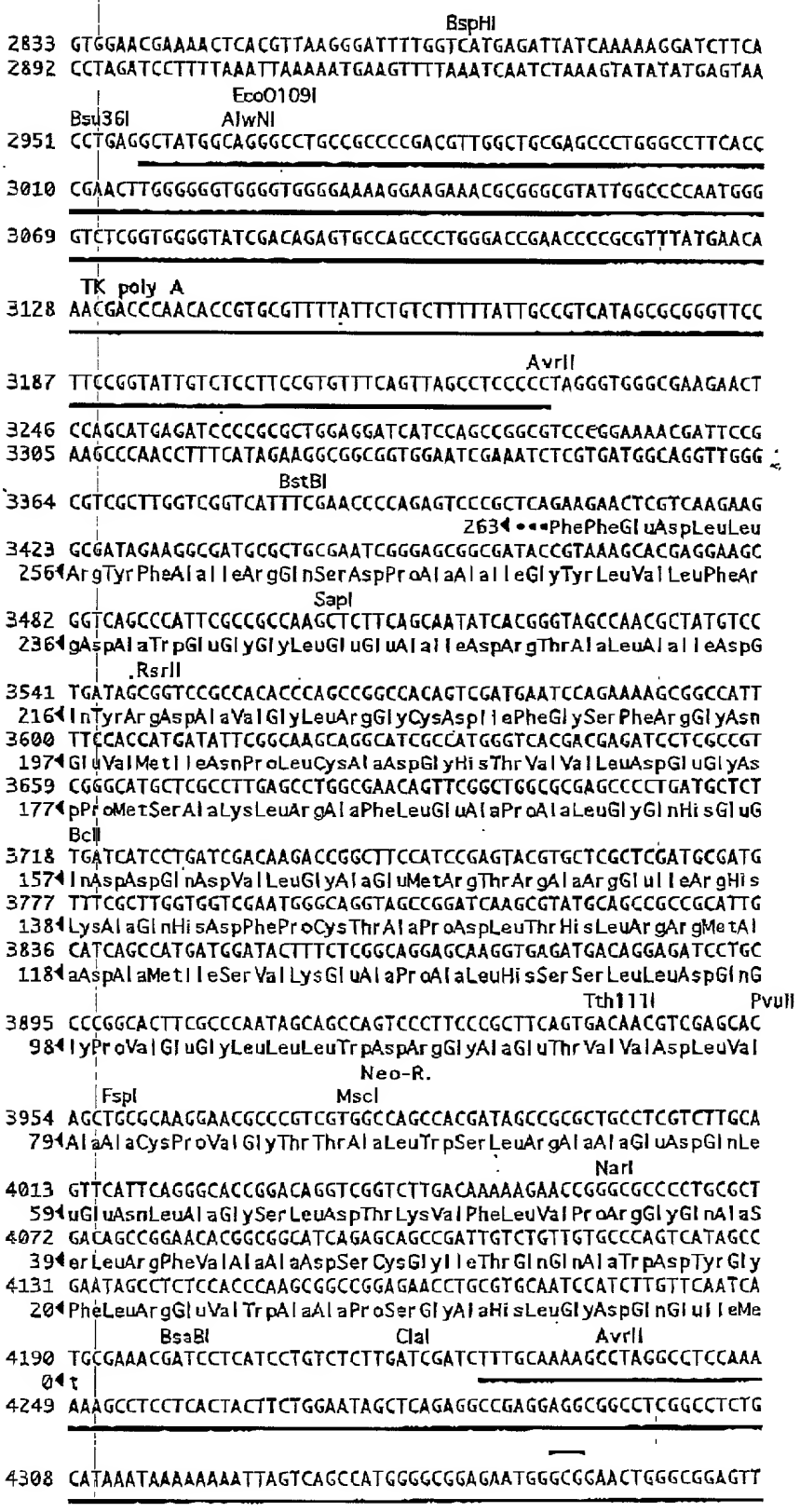

Fig. 1 (cont'd III)


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Stem loop A

Fig. (cont'd IV)

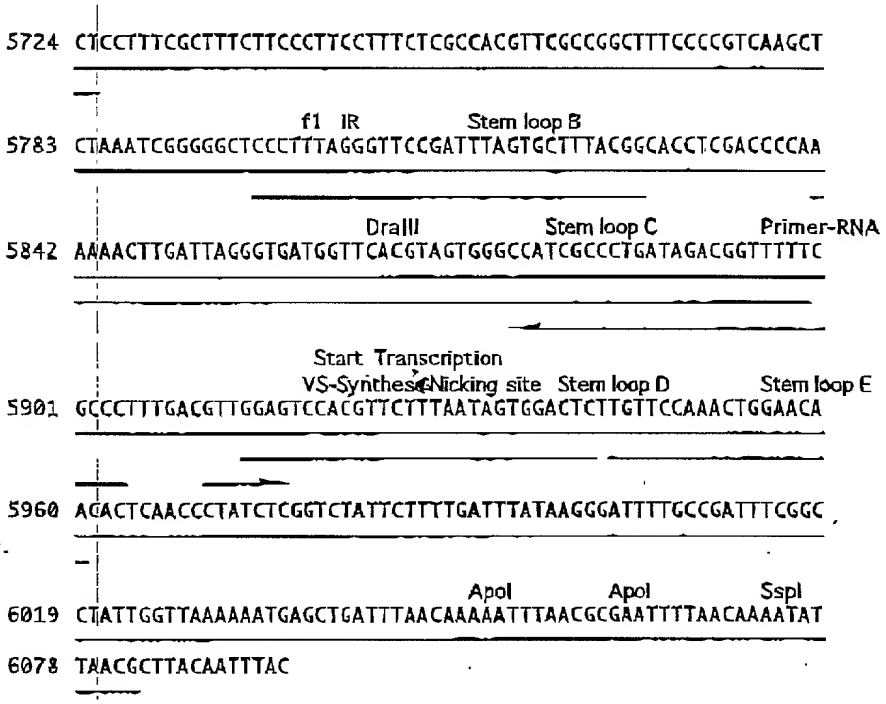

Fig. 1 (cont'd V)

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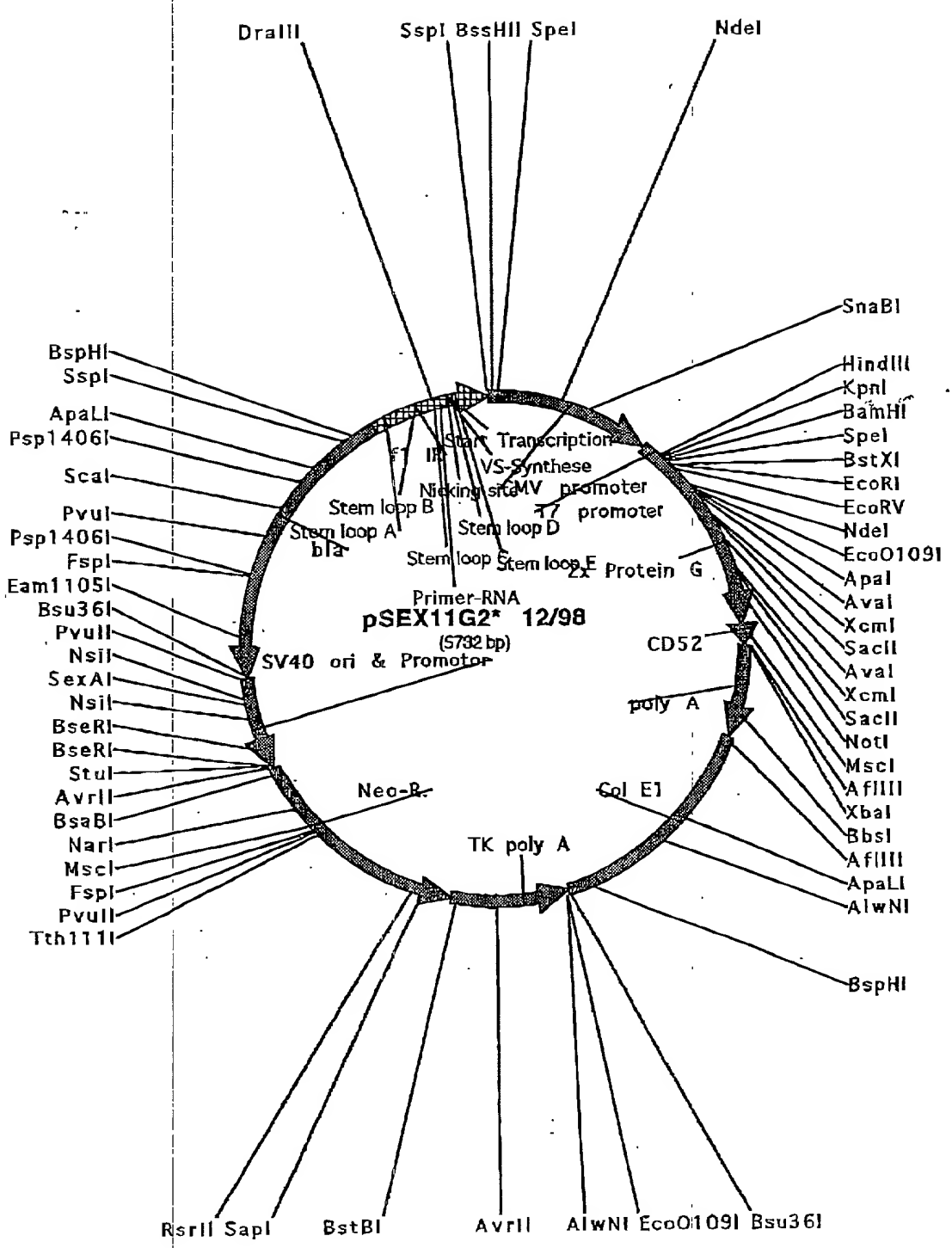
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Start Transcription
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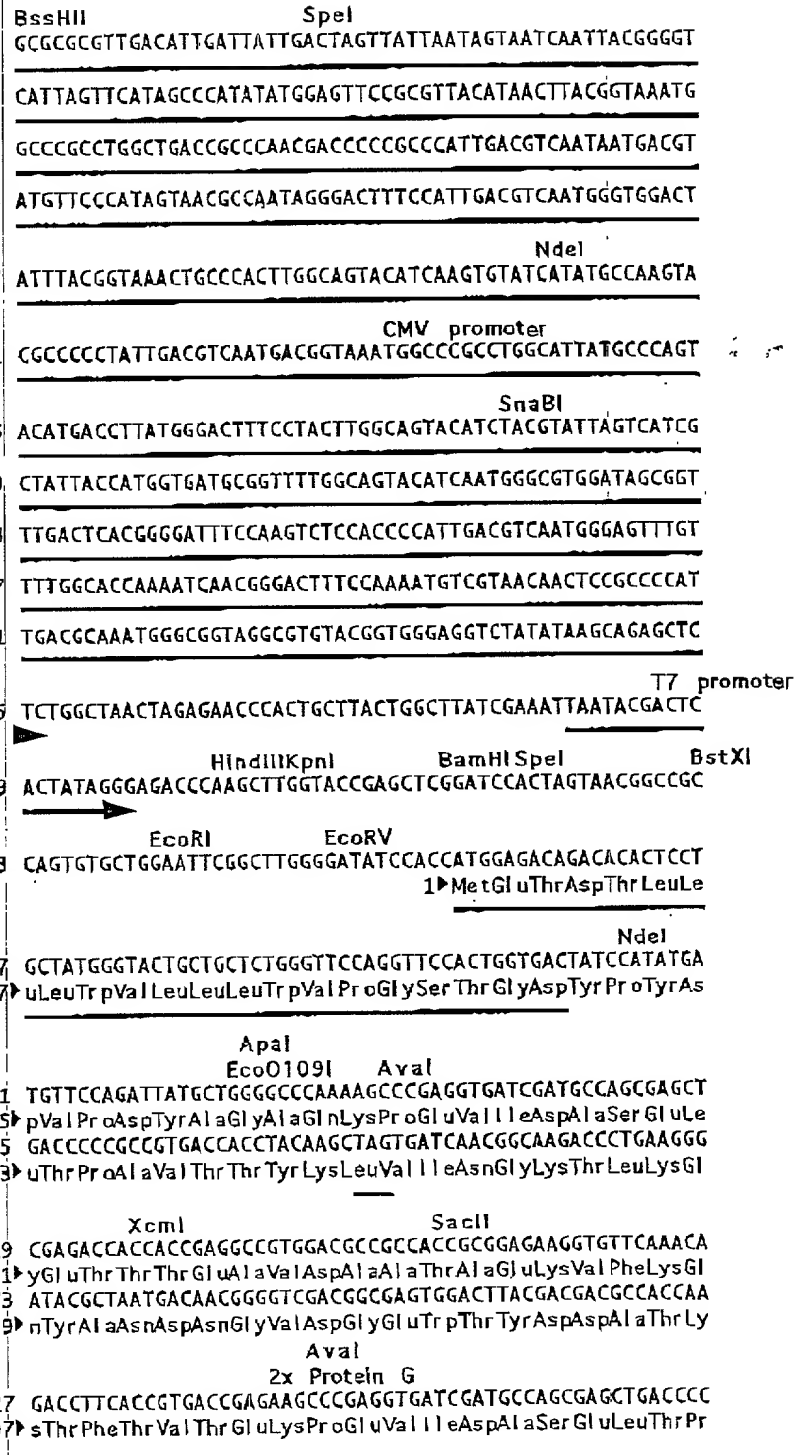
Nicking site
MV promoter
T7 promoter
Stem loop A
Stem loop B
Stem loop C
Stem loop D
Stem loop E
bla
2x Protein G
Primer-RNA
pSEX11G2* 12/98
(5732 bp)
SV40 ori & Promotor
CD52
poly A
Neo-R.
Col E1
TK poly A

Fig. 2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SpeI
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55 CATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATG
109 GCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGT
163 ATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACT
NdeI
217 ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTA
CMV promoter
271 CGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
SnaBI
325 ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCG
379 CTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGT
433 TTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGT
487 TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCAT
541 TGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTC
T7 promoter
595 TCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTC
HindIIIKpnI BamHI SpeI BstXI
649 ACTATAGGGAGACCCAAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGC
EcoRI EcoRV
703 CAGTGTGCTGGAATTCGGCTTGGGGATATCCACCATGGAGACAGACACACTCCT
1 MetGluThrAspThrLeuLe
NdeI
757 GCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACTATCCATATGA
7 uLeuTrpValLeuLeuLeuTrpValProGlySerThrGlyAspTyrProTyrAs
ApaI
Eco0109I AvaI
811 TGTTCCAGATTATGCTGGGGCCCAAAAGCCCGAGGTGATCGATGCCAGCGAGCT
25 pValProAspTyrAlaGlyAlaGlnLysProGluValIleAspAlaSerGluLe
865 GACCCCCGCCGTGACCACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGG
43 uThrProAlaValThrThrTyrLysLeuValIleAsnGlyLysThrLeuLysGl
XcmI SacII
919 CGAGACCACCACCGAGGCCGTGGACGCCGCCACCGCGGAGAAGGTGTTCAAACA
61 yGluThrThrThrGluAlaValAspAlaAlaThrAlaGluLysValPheLysGl
973 ATACGCTAATGACAACGGGGTCGACGGCGAGTGGACTTACGACGACGCCACCAA
79 nTyrAlaAsnAspAsnGlyValAspGlyGluTrpThrTyrAspAspAlaThrLy
AvaI
2x Protein G
1027 GACCTTCACCGTGACCGAGAAGCCCGAGGTGATCGATGCCAGCGAGCTGACCCC
97 sThrPheThrValThrGluLysProGluValIleAspAlaSerGluLeuThrPr

Fig. 2 (cont'd I)

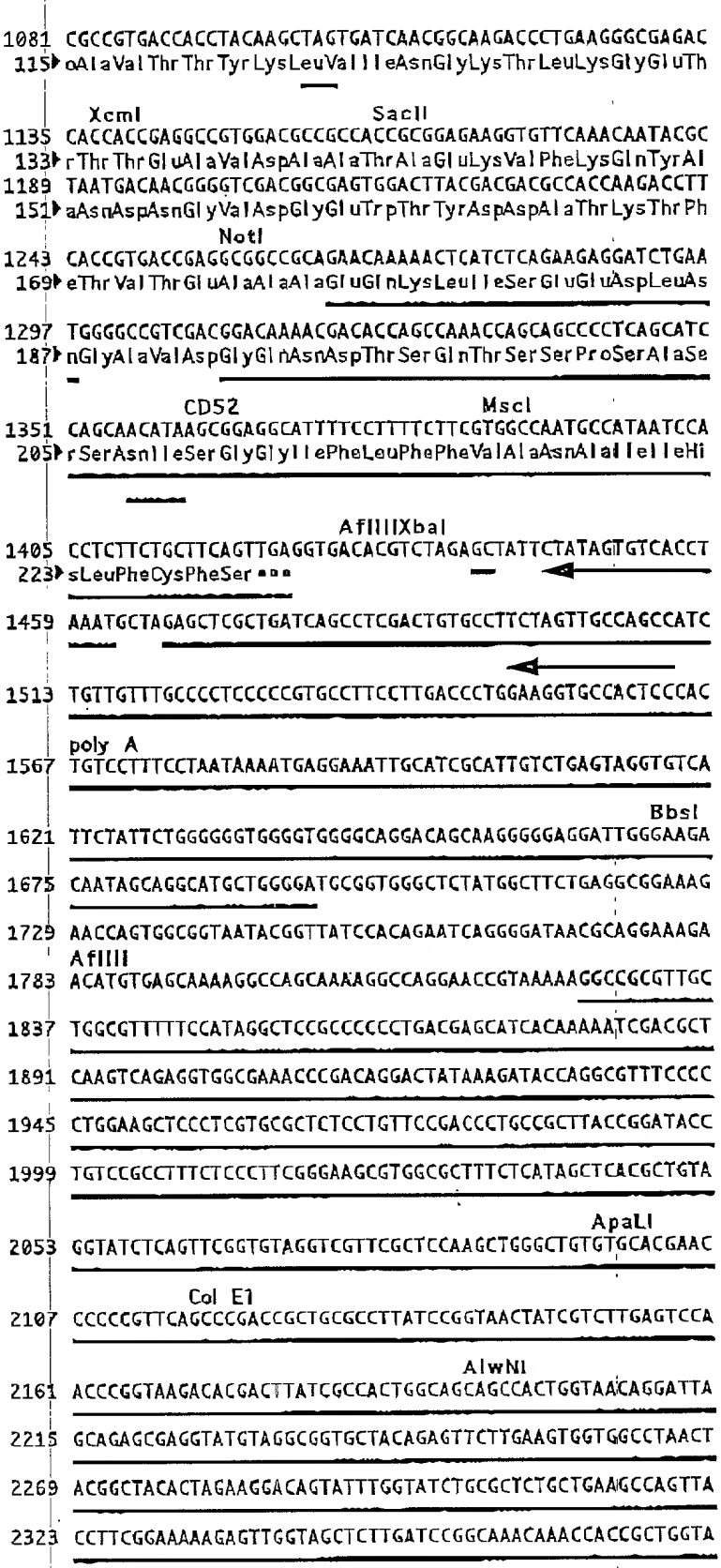


Fig. 2 (cont'd II)

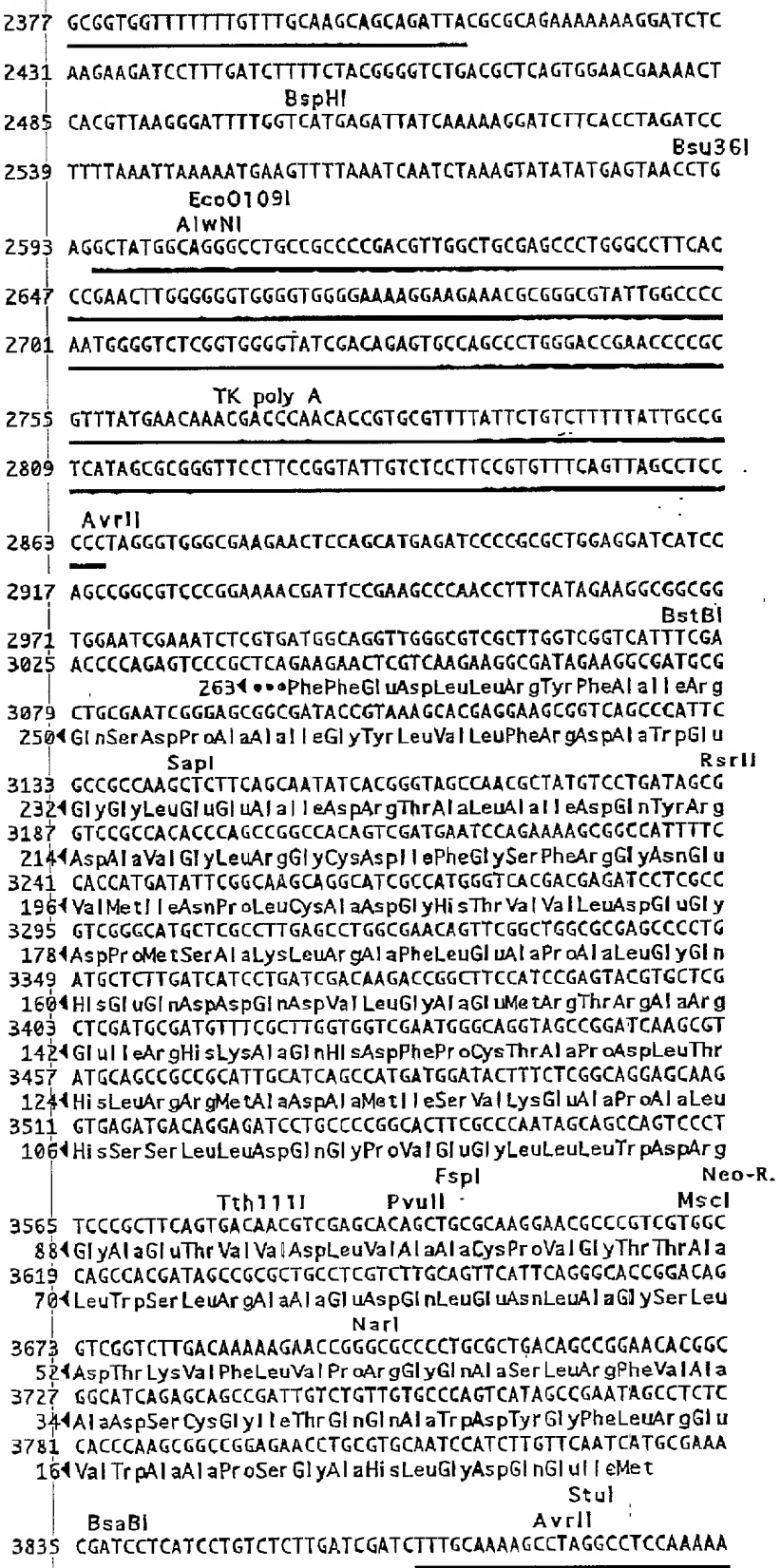
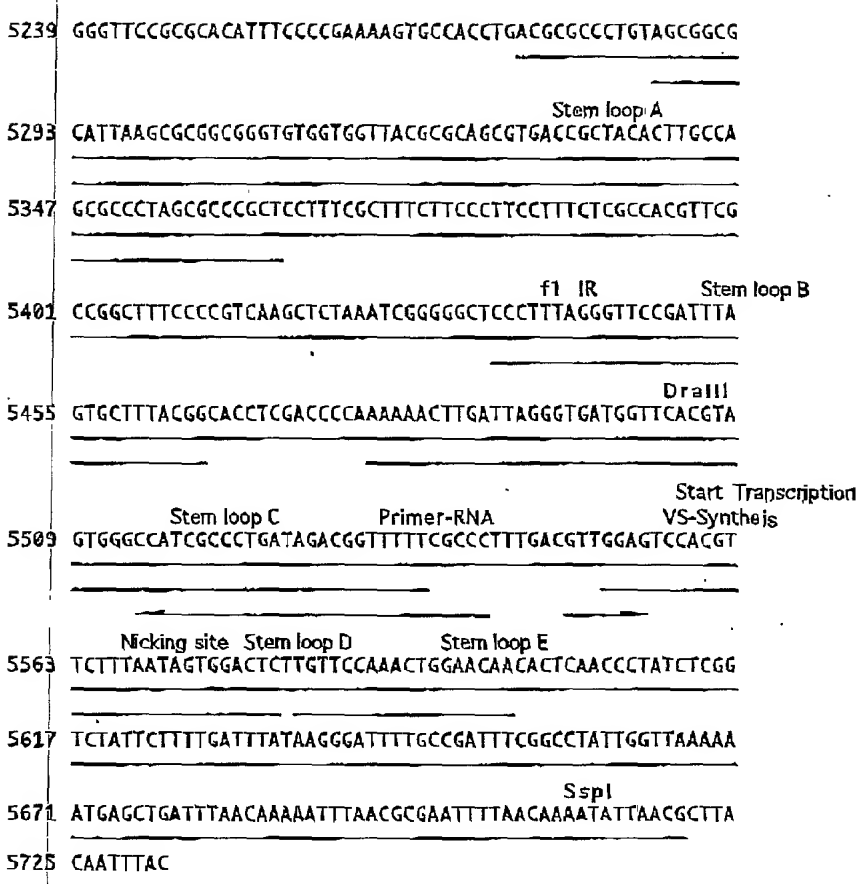


Fig. 2 (cont'd III)

Fig. 2 (cont'd IV)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Stem loop A
5293 CATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCA
5347 GCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCG
f1 IR
Stem loop B
5401 CCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTA
DraIII
5455 GTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTA
Start Transcription
Stem loop C
Primer-RNA
VS-Synthesis
5509 GTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGT
Nicking site
Stem loop D
Stem loop E
5563 TCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGG
5617 TCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAA
SspI
5671 ATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTA
5725 CAATTTAC

Fig. 2 (cont'd V)

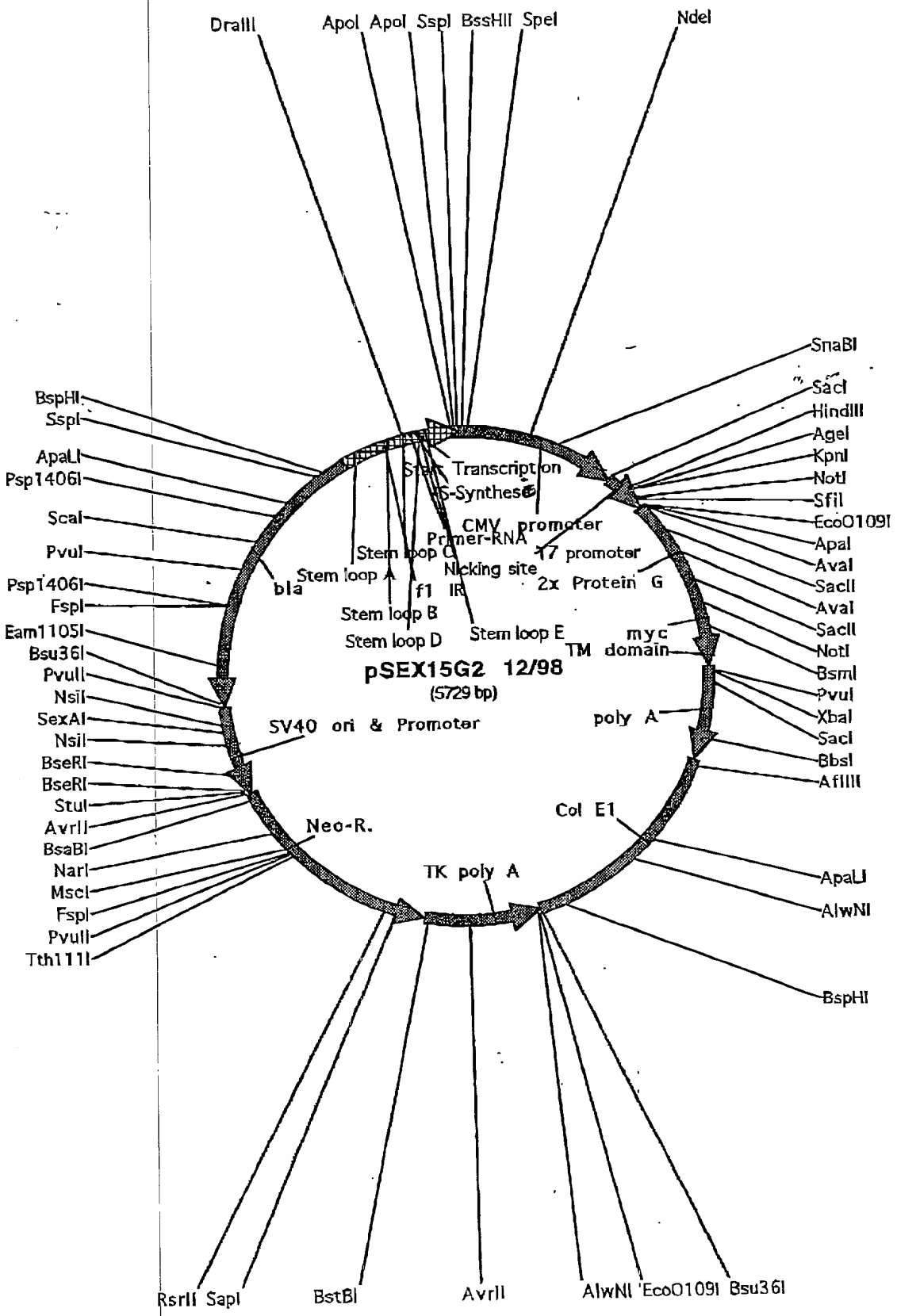
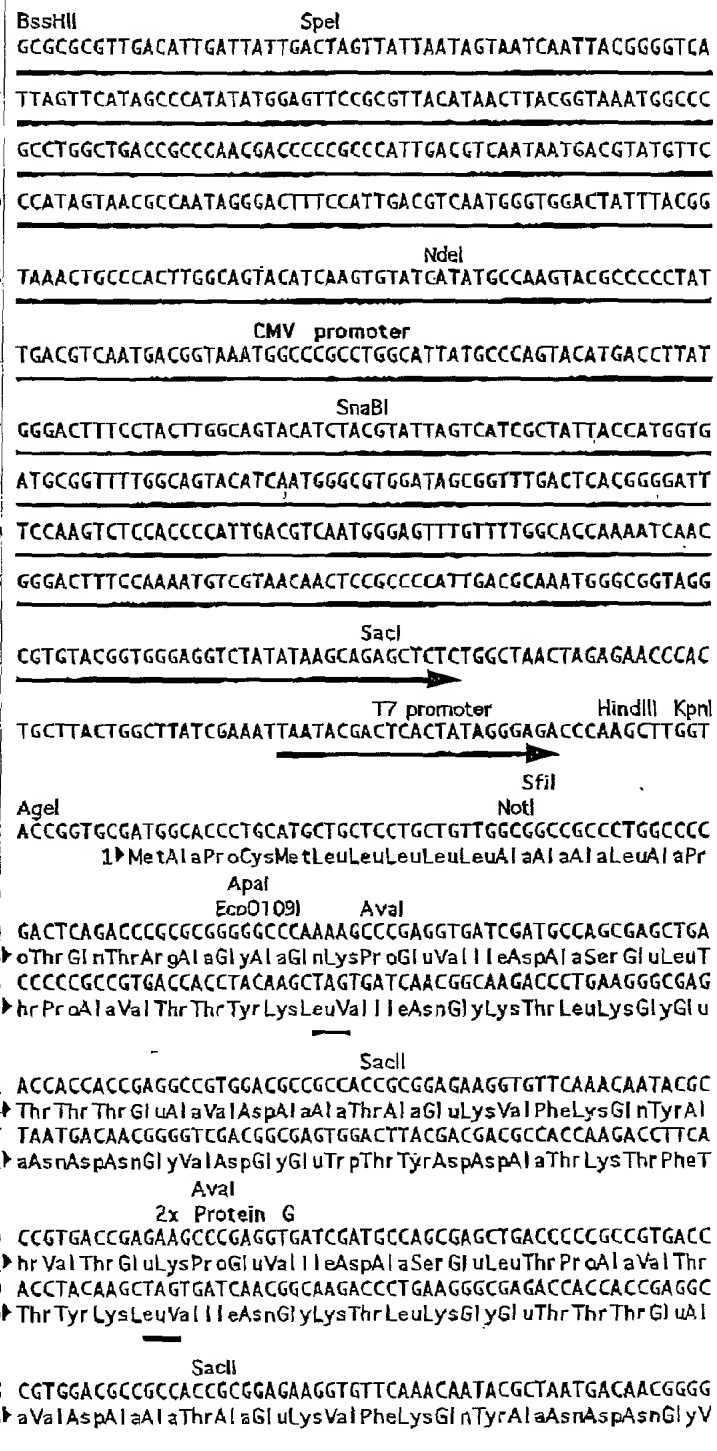

DraIII
ApoI ApoI SspI BssHII SpeI
NdeI
SnaBI
SacI
HindIII
AgeI
KpnI
NotI
SfiI
EcoO109I
ApaI
AvaI
SacII
AvaI
SacII
NotI
BsmI
PvuI
XbaI
SacI
BbsI
AflIII
ApaLI
AlwNI
BspHI
BspHI
SspI
ApaLI
Psp1406I
ScaI
PvuI
Psp1406I
FspI
Eam1105I
Bsu36I
PvuII
NsiI
SexAI
NsiI
BseRI
BseRI
StuI
AvrII
BsaBI
NarI
MscI
FspI
PvuII
Tth111I
RsrII SapI
BstBI
AvrII
AlwNI EcoO109I Bsu36I
Start Transcription
RNA-Synthese
CMV promoter
Primer-RNA
T7 promoter
Nicking site
2x Protein G
Stem loop C
Stem loop A
bla
f1 IR
Stem loop B
Stem loop D
Stem loop E
myc
TM domain
pSEX15G2 12/98
(5729 bp)
SV40 ori & Promoter
poly A
Col E1
Neo-R.
TK poly A

Fig. 3


BssHII
SpeI
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57 TTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCC
113 GCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTC
169 CCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGG
NdeI
225 TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTAT
CMV promoter
281 TGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTAT
SnaBI
337 GGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTG
393 ATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATT
449 TCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAAC
505 GGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGG
SacI
561 CGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCAC
T7 promoter
HindIII KpnI
617 TGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTTGGT
SfiI
AgeI
NotI
673 ACCGGTGCGATGGCACCCTGCATGCTGCTCCTGCTGTTGGCGGCCGCCCTGGCCCC
1 MetAlaProCysMetLeuLeuLeuLeuAlaAlaAlaLeuAlaPr
ApaI
EcoO109I
AvaI
729 GACTCAGACCCGCGCGGGGGCCCAAAAGCCCGAGGTGATCGATGCCAGCGAGCTGA
16 oThrGlnThrArgAlaGlyAlaGlnLysProGluValIleAspAlaSerGluLeuT
785 CCCCCGCCGTGACCACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGGCGAG
35 hrProAlaValThrThrTyrLysLeuValIleAsnGlyLysThrLeuLysGlyGlu
SacII
841 ACCACCACCGAGGCCGTGGACGCCGCCACCGCGGAGAAGGTGTTCAAACAATACGC
54 ThrThrThrGluAlaValAspAlaAlaThrAlaGluLysValPheLysGlnTyrAl
897 TAATGACAACGGGGTCGACGGCGAGTGGACTTACGACGACGCCACCAAGACCTTCA
72 aAsnAspAsnGlyValAspGlyGluTrpThrTyrAspAspAlaThrLysThrPheT
AvaI
2x Protein G
953 CCGTGACCGAGAAGCCCGAGGTGATCGATGCCAGCGAGCTGACCCCCGCCGTGACC
91 hrValThrGluLysProGluValIleAspAlaSerGluLeuThrProAlaValThr
1009 ACCTACAAGCTAGTGATCAACGGCAAGACCCTGAAGGGCGAGACCACCACCGAGGC
110 ThrTyrLysLeuValIleAsnGlyLysThrLeuLysGlyGluThrThrThrGluAl
SacII
1065 CGTGGACGCCGCCACCGCGGAGAAGGTGTTCAAACAATACGCTAATGACAACGGGG
128 aValAspAlaAlaThrAlaGluLysValPheLysGlnTyrAlaAsnAspAsnGlyV

Fig. 3 (cont'd I)

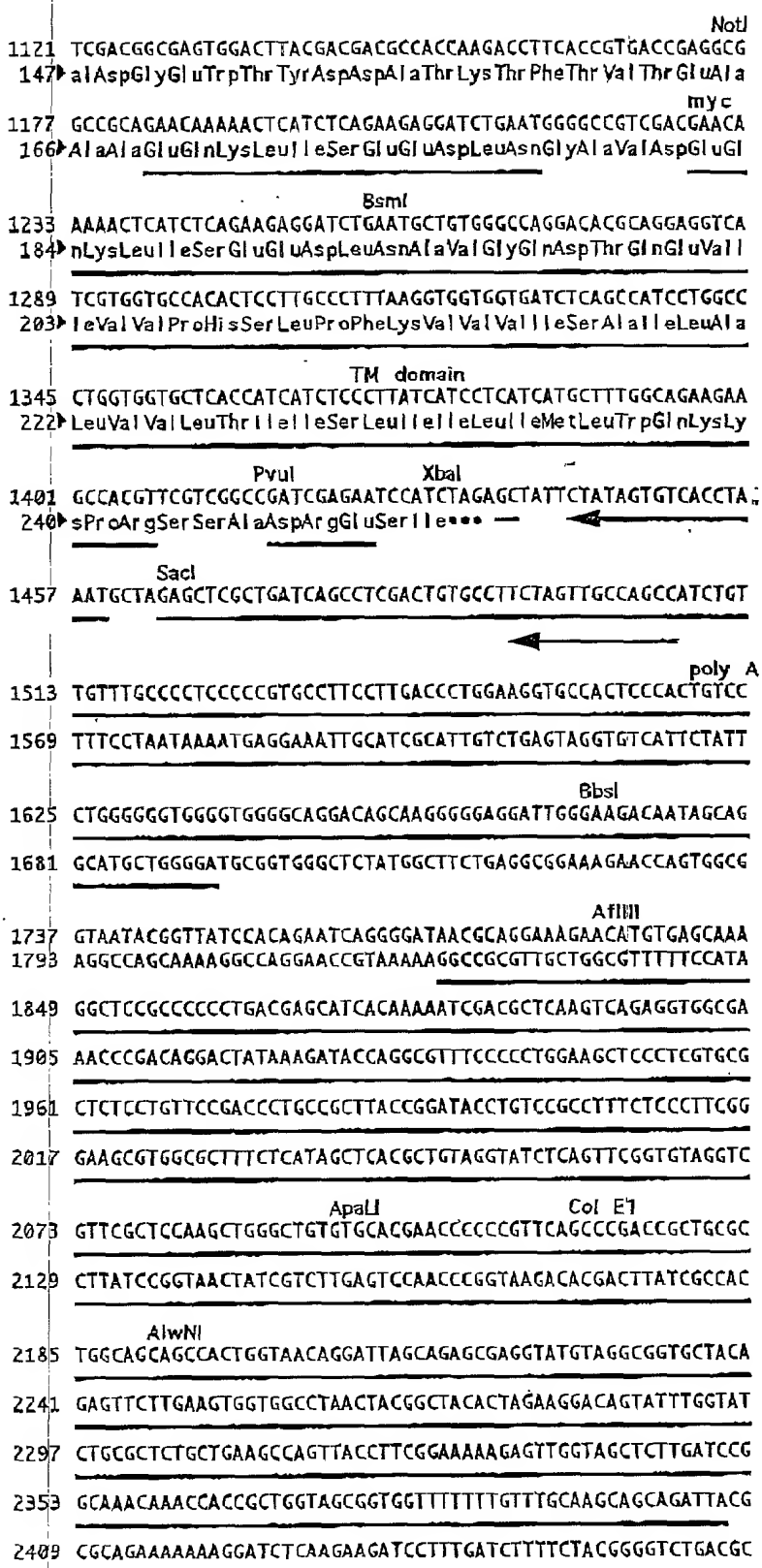

Fig. 3 (cont'd II)

Fig. 3 (cont'd III)

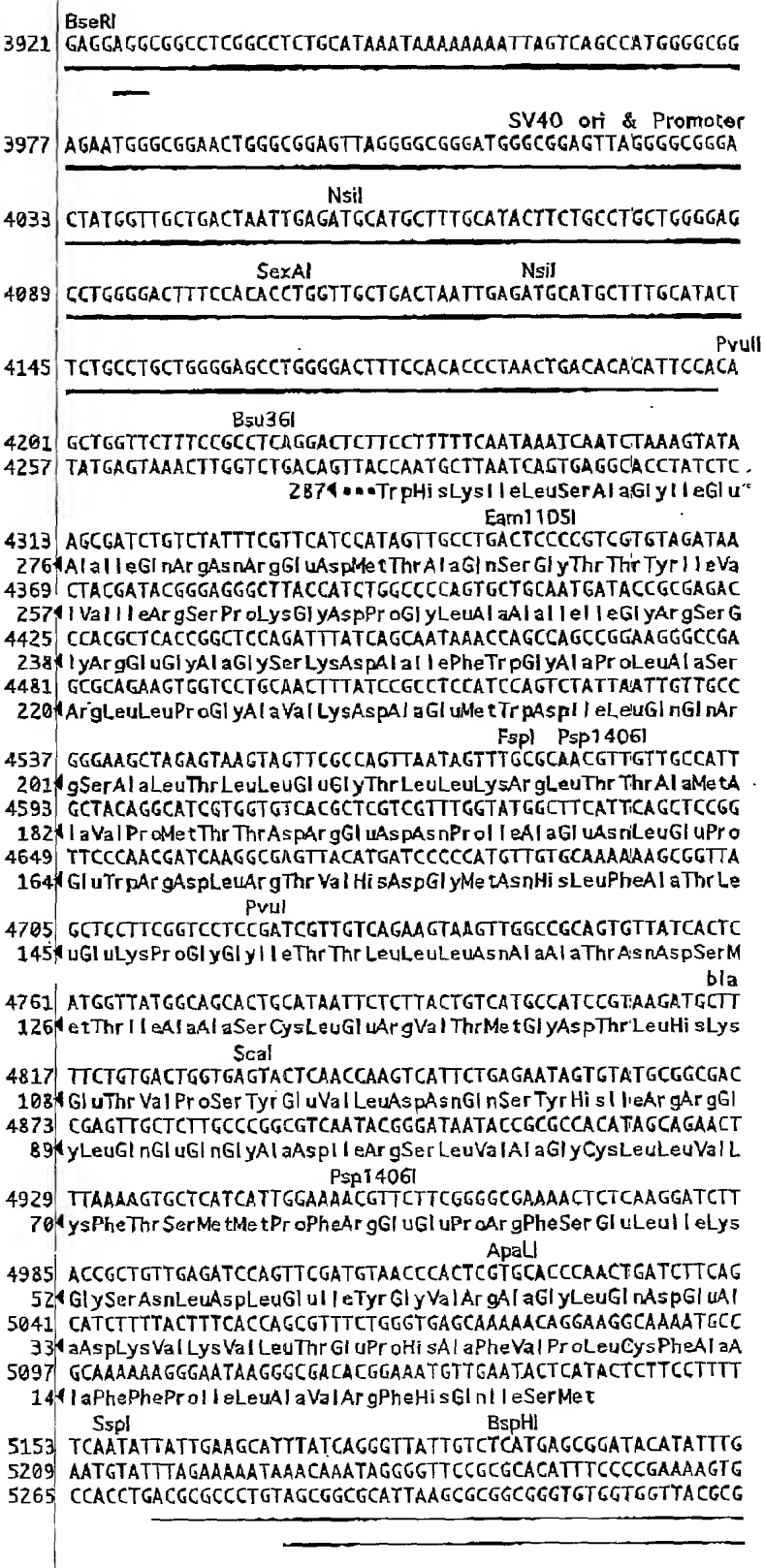

Fig. 3 (cont'd IV)

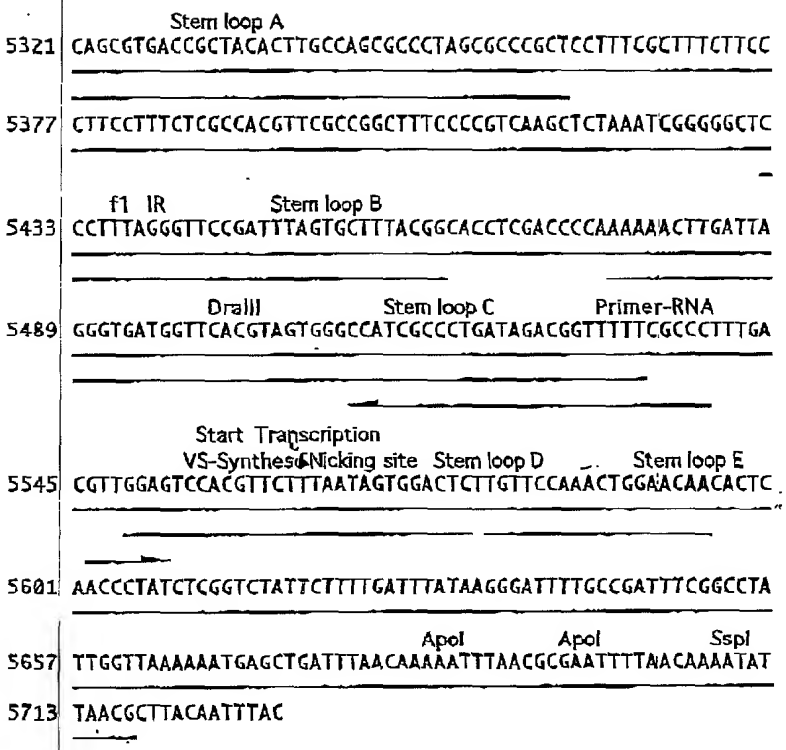
Stem loop A
5321 CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCC
5377 CTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTC
f1 IR
Stem loop B
5433 CCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTA
DraIII
Stem loop C
Primer-RNA
5489 GGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGA
Start Transcription
VS-Synthesis
Nicking site
Stem loop D
Stem loop E
5545 CGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTC
5601 AACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTA
ApoI
ApoI
SspI
5657 TTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATAT
5713 TAACGCTTACAATTTAC

Fig. 3 (cont'd V)